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Procedure, appliance and diagnostic agent for the detection of corpus luteum metabolic products

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The invention relates to a procedure, devices and diagnostic agents for the detection of corpus luteum metabolic products in the body fluids excreted by women for the purpose of determining the non-conceptive days of the women.

The known procedures for the detection of corpus luteum metabolic products were previously unable to be used for the determination of the conception-free days of the women, since certain amounts of these metabolic products are always present in the body fluids excreted by the women and detection reactions directed at these were considered as too unreliable for the purpose indicated.

The invention starts from the object of remedying the difficulty outlined and proposing a detection procedure for corpus luteum metabolic products which makes possible a reliable determination of the conception-free days of women.

The object is achieved according to the invention in that at least one corpus luteum metabolic product occurring in the body fluid is determined by means of reactions associated with a colour change and in that measures are taken here in order to detect the colour change only in the presence of those amounts of the metabolic product which originate from a functional corpus luteum.

An appliance preferably used for carrying out the detection procedure according to the invention is distinguished in that an outlet tube, which has a fine outlet opening for the body fluid to be tested, is removably attached at the bottom to a container containing the body fluid to be tested, and in that a tubular container which contains a solvent column and is connected at its lower end to an overflow tube is provided perpendicularly under the outlet opening, and in that finally the free opening of the overflow is so far below the level of the free outlet opening that the fluid jet emerging from the fine opening reaches the top of the solvent column in the form of a series of liquid drops.

A diagnostic agent for carrying out the procedure according to the invention is characterized in that it contains at least two substance mixtures in a total volume of at most 60 cc, of which one, on

contact with at least one corpus luteum metabolic product occurring in a body fluid of the woman, leads to a colour change, while the other is suitable to inhibit the colour change in such a way that only those amounts of the metabolic product are detectable which originate from a functional corpus luteum.

The ovulation date was regarded until now as a criterion in the determination of the non-conceptive period in women. Accordingly, all methods known to date for the determination of the non-conceptive period are directed at a determination of ovulation as such which is as accurate as possible. According to the most recent knowledge of the inventor, however, ovulation is meaningless in relation to the non-conceptive period, because the biological sterility of women which in general follows an ovulation is not a result of the ovulation, but one of the corpus luteum forming in the implanted graafian follicle. For this reason, not ovulation, but the functionability of the corpus luteum, is detected according to the invention in the determination of the non-conceptive period.

As is known, the corpus luteum produces progesterone, which exerts the following important functions in the course of the menstrual cycle in women:

1. it inhibits further maturation of the ovum in the ovaries;
2. it paralyses the uterine musculature as protection for the
3. endometrium which is changing and "growing" in the secretory state under its influence, and
4. as a further protective function for the endometrium, it increases the viscosity of the cervical mucous plug in the cervix.

These four genital functions, however, are only guaranteed if the progesterone production of the corpus luteum reaches an adequately high level.

In the case of inadequate progesterone production or a not completely functional corpus luteum

(which can be the case with or without external influences, such as, for example, health cures, changes of climate, diseases etc.), the inhibition of the maturation of the ovum in the ovaries is not always guaranteed as the first thing. Within the next 8 to 10 days a second follicle which is ready to burst is then formed in the same menstrual cycle, which bursts immediately before the menstrual period which customarily follows during the normal decrease in the amount of progesterone, which per se is already too low. A premenstrual secondary ovulation results, which can lead to conception in the so-called "non-conceptive" period. The second corpus luteum forming in the secondary ovulation prevents the occurrence of the menstruation which is customary at this point in time and shifts this up to its degeneration by 12 to 14 days if the secondary ovulation has not led to fertilization. Of course, the premenstrual secondary ovulation can also occur in a completely functional corpus luteum; fertilization, however, is then not possible, because in the case of sufficiently high progesterone production the uterine mucosa are in the grown state and the cervical mucus is of increased viscosity, so that these two natural barriers block the penetrating spermatozoa from entering and passing through the uterus and as a result ascending to the ovaries.

These connections were found experimentally under practical testing by a relatively large number of married couples. Premenstrual secondary ovulations resulted here, both in the case of inadequate and adequate corpus luteum activity. Pregnancies arose, however, only in the first case, while the second type of secondary ovulations remained without consequences.

Measured on the most important excretion product of progesterone, pregnanediol glucuronide, which additionally contains 25% of pregnanolone in its complex as an "impurity", it turned out on the basis of these empirical experiments that in the average case it

is possible to speak of the presence of a functional corpus luteum with a daily excretion of 3.5 mg of pregnanediol glucuronide (including pregnanolone) and in some isolated cases of at least 2.5 mg. This relationship agrees well with the already known facts that during the proliferation phase of the endometrium 1 to 2 mg of pregnanediol glucuronide per day are excreted in the urine of women, which minimum amounts originate from the corticoadrenal metabolism or premature luteinisation of the follicle. On the other hand, as known, the normal amounts of pregnanediol glucuronide excreted in the actual corpus luteum phase are on average 8 to 9 mg per day.

Below the abovementioned threshold value of 3.5 or 2.5 mg of pregnanediol glucuronide per day, fertilizable secondary ovulations result, because the simultaneous growth of the uterine mucous membrane and the increase in the cervical mucus viscosity are incomplete and cannot act as barriers, whereas secondary ovulations above this threshold value are no longer fertilizable.

On consideration of the threshold values mentioned on the other hand, however, not only the functionability of the corpus luteum can be detected, but at the same time the small amounts of progesterone or pregnanediol originating from the corticoadrenal metabolism (conversion of the deoxycorticosterone into progesterone) and the amounts already ubiquitously produced preovulatively in the unburst follicle are also "reconciled". The previously known procedures, be they now for progesterone or pregnanediol glucuronide determination, and further procedures for the determination of other degradation products of progesterone, can be provided with sensitivity thresholds which are equivalent to the threshold values mentioned further above. Thus, those purely quantitative substance determination procedures automatically become qualitative procedures for the determination of the non-conceptive period if the

procedure time needed for carrying them out is not too long, in order to keep the reaction so early that a satisfactory utilization is still possible.

The introduction of such a threshold value into any known procedure can be simply achieved in that, for example, the obviousness of a colour reaction is raised up to the prescribed limit. This can be achieved in that either, as a result of restricted stoichiometric reaction, only so much of the reacting substance is absorbed in the colour reaction that it responds only at the threshold value as a result of its intrinsic sensitivity limit (e.g. use of poor solvents in order that only part of the reacting substance goes into solution), or else in that the colour reaction itself is inhibited by chemically or physically acting additives. Such additives can be oxidizing or reducing (bleaching) and also complex-forming substances which mask the corresponding colour components, or alternatively adsorbent or precipitating agents which in themselves take up or precipitate the threshold component, or complementary colours which compensate the threshold component.

The procedures for the determination of progesterone in the blood or its degradation products and especially pregnanediol glucuronide in the urine known today are virtually unusable for birth control and are suitable only for clinical diagnostic purposes. They are involved in every respect and require expensive equipment and highly qualified personnel. Moreover, all of them produce the results with so great a delay that practical application cannot be considered at all without significant modifications. The procedure according to the invention avoids these disadvantages. As the detection of the non-conceptive period does not necessitate any quantitative hormone determination, but only the determination of a minimum hormone level in a semiquantitative manner, a simple procedure which can be carried out rapidly has been developed.

The procedure can be carried out so simply and the expenditure of time needed for carrying it out are so low that it is suitable not only for the laboratory equipped for its clients especially for the purpose of determining the non-conceptive period, but moreover can be used by any unskilled people or any woman herself at home. Whereas in the laboratory any apparatus which is adapted to the procedure requirements, in particular the extraction of the metabolic products from the body fluid, is suitable for unskilled people a specially small-sized device which makes possible schematic operation according to use instructions is indispensable. For the rapid and reliable carrying-out of the procedure according to the invention by unskilled people, the diagnostic agent, i.e. the necessary chemicals, solvents, reagents, dyes etc., is expediently prepared as a pack measured ready-for-use for one determination each and which can be kept as long as possible. These packs consist, for example, of ampoules, tubes and the like made of glass or plastic and also plastic tubing etc. sealed off in the form of portion packs. Such combined packs for one determination each not only save the troublesome measurement of the individual portions, but also guarantee greater durability than, for example, in the case of bottles which are opened again and again.

Such a combined diagnostic agent according to the invention in packed form weighs, for example, 30 g and has a volume of approximately 34 cc; it can be combined to give annual packs for twelve to twenty determinations having a total weight of around 500 g. The individual diagnostic agent for one determination does not quite weigh 16 g and has a volume of around 19 cc, i.e. significantly less than in the case of all known pregnanediol glucuronide determination procedures, of which not a single one can manage with a total of less than 60 to 70 cc of chemicals.

The implementation of the procedure according to the invention is described purely for example below

in an embodiment preferred for the pregnanediol glucuronide-pregnanolone complex:

Fig. 1 shows an extraction apparatus belonging to the device in the operating state;

Fig. 2 shows a complete device boxed one inside the other in the non-operating state;

Fig. 3 shows a shaking beaker for the wash liquids with an attached cover;

Fig. 4 shows a vessel holder with a reaction vessel, a burner and a condenser tube in the operating state.

The procedure consists essentially in extracting the pregnanediol glucuronide complex from the fresh urine, which is excreted during the course of 8 to 10 hours and then saturated with sodium chloride, washing the extract, subsequently precipitating the pregnanediol glucuronide-pregnanolone complex directly as a barium salt and then investigating it as a precipitate for its glucuronic acid content with the aid of Tollens reaction. Both in the extraction stage, the washing of the extract, the precipitation and the colour reaction according to Tollens, intentional inhibition stages are incorporated into the procedure which reduce its sensitivity to the threshold value mentioned further above. Reference is later made in greater detail to these inhibition stages.

The extraction apparatus (Fig.1) expediently to be used by unskilled people consists of a basic vessel **1** and an extractor **2** inserted therein (tube diameter advantageously 10.5 mm, not over 20 mm), which hermetically seals to the base vessel **1** by means of seals **9**. A nozzle piece **3** (diameter advantageously 0.58 mm) is screwed on the extractor **2** via a seal **14** and a top vessel **4** is screwed onto this via a seal **18**. In the top vessel **4** is inserted a sieve **7** held by a tension spring **6**, and the whole is closed with a mounted cap **5**. In the upper part of the extractor **2** is located a pump consisting of a piston **15**, a stop screw **16**, a pressure spring **17** and a valve tube **25**, and a valve consisting

of a valve head **12**, a valve disc **11**, a seal **10** and a passage opening **13**. Attached to the side of the extractor **2** is an overflow **2a** and at the very bottom a closing cover **8**. In the upper part of the nozzle piece **3**, i.e. directly above the nozzle, a regulating valve can be attached in order to regulate the pressure of the jet of urine flowing through the nozzle; this valve, however, is not absolutely necessary and is therefore left out in the drawing.

In the non-operating state of the device (Fig. 2) the top vessel **4**, the nozzle piece **3** and all other components are accommodated in the interior of the base vessel **1**. The device then has only around one third of its operating height. The cover **5**, whose seal **19** is intended to seal a shaker beaker **23** (fig. 3), is then screwed into the upper part of the extractor **2**, where the nozzle piece **3** is fixed in the operating state. The valve **12** is closed and the pump piston **15** is held down by the cover **5**. Two wire clamps **24** clamped onto the extractor **2** are intended to retain the two reaction vessels **21**, e.g. of fire-resistant glass (of which only one is shown in section in Fig. 2), and the nozzle piece **3** in the interior of the base vessel or of the top vessel **4**. At the very bottom, the shaker beaker **23** is fixed by the widened calming part of the extractor **2**, below a burner **24** and in addition a vessel holder **20**. A condenser tube **26** (Fig. 4) and the sieve **6,7** are, because they are elastic and therefore accommodatable everywhere in the interior of the device, not indicated.

The base vessel **1** serves as a collection vessel for the urine and for its saturation with sodium chloride. The amount of urine from an excretion time of 8 to 10 hours is made up with tap water to the mark on the base vessel **1** (Fig. 1), which corresponds to an amount of 700 cc, which amount is rarely exceeded within 8 to 10 hours. So much sodium chloride is added to this that the liquid level rises from the lower to the upper mark (770 cc). Extractor **2** with the screwed-

on covers 5 and 8 is then inserted into the base vessel **1** so that this is hermetically sealed by the seals **9**. After vigorous shaking lasting for 40 seconds, the urine is saturated with the added sodium chloride.

During the shaking, the extractor **2** is also filled with urine by the adjacent overflow **2a**. After unscrewing the cover **5** to expose the upper opening of the extractor tube **2**, the valve **12** consisting of the disc **11**, seal **10** and the opening **13** is opened. This allows for the escape of the air in the subsequent extraction, in which the base vessel **1** slowly fills. After filling of the extraction fluid contained, for example, in one portion pack of plastic tubing, consisting of 9 cc of amyl butyl alcohol in the volume ratio 1:2 into the extractor tube **2**, a solvent column is formed in this which forms a layer on the salt-saturated urine; as a result of the overflow, the solvent always adjusts to the same level, namely approximately 4 cm below the nozzle. After filling, the nozzle piece **3** is screwed onto the extractor **2** and the top vessel **4** is screwed onto this and into the latter is placed the sieve **6,7**. After this, the base vessel **1** filled with salt-saturated urine is taken off and emptied into the top vessel **4**. The base vessel **1** is immediately attached again at the bottom, since urine soon escapes through the overflow; the cover **5** is placed on the vessel **4** and the apparatus is put aside.

The amount of urine of around 770 cc then flows for approximately 35 minutes in a fine jet through the nozzle and impacts on the surface of the amyl butyl alcohol in the extractor at that position where the jet begins to break up into fine droplets (flow quantity approximately 22 cc per minute). The urine is broken here into many minutely small droplets, which slowly fall through the solvent layer, combine under this again with the already extracted urine, ascend through the overflow **2a** and flow back into the base vessel **1**. The extraction of the pregnanediol glucuronide from the urine takes place here.

After the passage of the urine, the vessel **4** and the nozzle piece **3** are unscrewed, rinsed with water and attached again. The washing fluid necessary for the extract washing, consisting of 5 cc of ammoniacal ether-butanol mixture (from a portion pack made of plastic) in the volume ratio 5:1 and 0.5 cc of water with 200 mg of sodium chloride dissolved therein, is vigorously mixed in the shaking beaker **23** closed by the cover **5** (Fig. 3) and emptied into the top vessel **4**. The washing liquid then passes, similarly to the urine in the extraction, through the extract phase, and washes this so that the greatest part of the impurities is washed out of the extract. As a result of the ether character of the wash liquid and the small addition of sodium chloride, the pregnanediol glucuronide in the majority of all cases does not pass over into the washing liquid. There are meanwhile, however, exceptional cases with extremely slightly contaminated urines: the impurities are already removed from the extract in the first moment of washing, so that the greatest part of the washing liquid passes through the extract without further absorption of substance and for this reason can wash out the pregnanediol glucuronide despite the ether and sodium chloride content. In such cases of chronically slightly contaminated extracts, it is recommended in each case to use only two thirds of the around 40 cc of mixed washing liquid or in extreme cases only half. Rewashing with 35 to 40 cc of saturated brine for the purpose of dewatering of the turbid extract and removal of excess ammonia then takes place. The extract is then usually colourless and clear.

After the deposition of the last, finest brine droplets in suspension (2 to 3 minutes after washing with brine), nozzle piece **3** and top vessel **4** are unscrewed and the valve **12** is closed. The urine level in the base vessel **1** is now somewhat above the overflow opening **2a** as a result of the volume increase due to the washing liquids. An appropriately shaped plunging

syphon (not shown in the drawings) is pressed onto the seal **14** and the pump plunger **15** is actuated with the index finger of the other free hand. The finger pressing on the plunger is at the same time an inlet valve, and the air forced into the base vessel **1** is prevented from escaping by the valve tubing **25**. As a result of the excess pressure generated in the interior of the vessel **1**, the extract phase ascends in the extractor until it is completely in the plunging syphon resting on the seal **14**. Its free opening at the upper end is then kept shut with the finger and the extract is emptied into one of the small reaction flasks **21** made of fireproof glass, which must be dry inside. Instead of using excess pressure through the pump 15, the extract can of course also be sucked out of the extractor **2** by means of pipette and suction valve. The delicate pump device could thus be superfluous.

Inspite of its colourlessness, the washed extract is still relatively highly contaminated. For this reason, the pregnanediol glucuronide is advantageously precipitated directly from the extract as the barium salt, which is not conventionally possible because of the solubility and concentration conditions. To this end, 0.3 g of silica gel of 0.2 to 1.0 mm grain size, which is impregnated with around 28 mg of barium acetate by hydration in distilled water containing 100 g of barium acetate per litre and subsequent drying, are added to the extract from the corresponding bag of diagnastic agent. The extract and silica gel are moderately shaken for approximately 1 minute. In this procedure, a part of the residual moisture remaining in the extract is first deposited on the silica gel and forms a water sheath around this, which contains the above barium salt dissolved in a very high concentration. Almost simultaneously, almost all substances present in the extract are adsorbed by the moist silica gel by passing them through the barium salt/water sheath. In this passage, the sodium pregnanediol glucuronide is precipitated momentarily as

barium pregnanediol glucuronide and as a result of the mechanical action of the shaking is immediately washed back into the extract from the water sheath. This operation, which is made up of an adsorption, almost simultaneous precipitation and quasi-desorption and theoretically completes the conventional stages of adsorption, elution, drying, redissolution, precipitation etc. in a single stage, is designated below as adsorption precipitation.

The suspension crystallizate is emptied into a second dry bulb **21** with the liquid phase leaving behind the silica gel and, to accelerate the deposition of the suspension 0.5 cc of gasoline, petroleum ether, benzene etc. and 0.1 cc of N/1000 hydrochloric acid is added from a further portion pack of the diagnostic agent and mixed by very vigorous shaking. Owing to this, the solid crystal constituents become wet and "tacky" on their surface; they mutually deposit on one another and, owing to this and as a result of the increase in weight caused by the moisture, fall relatively rapidly, (within 10 to 20 minutes) into the base of the bulb, where they likewise stay stuck as a result of their moisture content. By means of this, simple emptying of the mother liquid without stirring up the precipitate becomes possible. The hydrochloric acid content of the moisture prevents adsorption of uronic acid impurities on the precipitate.

The supernatant liquid is then emptied off, the bulb **21** is allowed to stand inverted for a short time in order to make possible residue-free emptying of the extract liquid, and the residue sticking to the bulb wall is taken up in tap water, which is poured in up to the graduation mark of the bulb **21** (Fig. 4). 1.5 cc of pure, concentrated hydrochloric acid (specific weight 1.19) and 0.5 cc of ethyl or propyl alcohol (pure) containing 3 mg of naphthoresorcinol are added to this water from further portion packs of the diagnostic agent. If the naphthoresorcinol solution is to be made storable for at least one year, the alcohol is

deaerated (by boiling with reflux condensation) and after this saturated with CO_2 before dissolving the naphthoresorcinol.

To put down the flask **21**, use is made of the flask holder **20** (Fig. 4), which together with the inserted flask **21** forms a securely standing tripod, as it has a broad, two-toed foot at its end. If no other flame is available, the burner **22** (Fig. 4), consisting only of a little bowl, is filled with alcohol, methylated spirit, a Meta tablet, eau de cologne etc. or, if every other fuel is lacking, is filled with the extract phase on pouring off (which, however, smokes somewhat on burning) and ignited. The condenser tubing **26** is mounted on the flask **21** and the reaction mixture is heated over the flame for one minute after the start of boiling. The vile-smelling vapour escaping from the flask on boiling condenses in the course of this in the condenser tubing **26**, so that the whole reaction proceeds virtually without odour. This destruction of odour by condensation is of importance, since without it use by unskilled people, i.e. in living rooms, would in many cases be made impossible.

After the cooling of the reaction mixture, the condenser tubing **26** is removed from the flask **21** in the horizontal position in order to avoid running-out of the condensate. From the last portion pack of the diagnostic agent, 1.5 cc of benzene, toluene, or xylene which has been coloured slightly green with 4 mg of Aniline Oleogreen per litre are added and the entire mixture is vigorously shaken for 15 to 20 seconds.

The red-violet colouration extracted from the benzene layer seperating out at the top is the indication of the non-conceptive period. If the solution remains green, water-clear or yellow to brownish, either no corpus luteum activity at all or an inadequate corpus luteum activity and consequently danger of conception is present. In this case, the analysis is repeated 2 to 3 days later until the red-violet colouration appears. Normally, carrying out the

determination once or twice monthly is completely sufficient in practice, the first determination falling on the 17th to 18th day of the cycle in, for example, a 28- to 30-day cycle. The actual working time for carrying out the determination is only around 15 minutes and its entire implementation time is approximately 1 hour (compared with a few hours working time and a few days implementation time in known procedures for pregnanediol glucuronide determination). The preovulative non-conceptive period is calculated as previously on the basis of the method according to Knaus Ogino, it becoming evident in practice that the reliability is only insignificantly modified by this combination, because bringing forward of the ovulation very rarely occurs.

Compared with the previously known procedures, the present procedure differs especially with respect to its extraordinary specificity and its relative sensitivity, which allows the use of less than one third of the daily urine output. The extremely low expenditure of time allows the onset of the non-conceptive period to be determined just 8 to 9 hours after the start of the first pregnanediol glucuronide formation in the female body. Thus in practice, a delay relative to the biological onset of the sterile phase can no longer be spoken of.

The manner of extraction in a thin jet is due to the extremely small amount of solvent used of only 9 cc or one eighty-fifth of the urine output or around one tenth to one twentieth of the known procedure. In the conventional type of extraction (shaking, compressed air injection, stirrer etc.) the small amount of solvent would completely dissolve even in approximately 500 cc of salt-saturated urine under the mechanical action. The addition of amyl alcohol to the butyl alcohol reduces the solubility for pregnanediol glucuronide and consequently constitutes a first stage in the total threshold value.

Compared with conventional types, the type of washing by, for example, shaking twice with one third each of the extract volume of N/3 to N/10 sodium hydroxide solution has around a thirty times higher efficiency, which is caused on the one hand, by the amount of washing liquid exceeding the extract volume by several times and on the other hand by the ether character of the washing water, the latter of which in turn strongly counteracts a transition of the pregnanediol glucuronide into the washing fluid. However, since it is impossible to prevent the transition of a small portion of pregnanediol glucuronide into the washing fluid, this residual transition constitutes a further part of the total threshold value.

The operation which is most important and improves and also shortens the procedure the most is adsorption precipitation. It can be designated as catalytic precipitation, the adsorbent playing the role of the catalyst. Apart from the significant simplification of the method, it offers the advantage that the precipitation becomes a decidedly time-dependant operation: only the most rapidly and most easily precipitating substance succeeds it, as all possibly more slowly precipitating substances are firmly bound to the silica gel even before their precipitation. Adsorption precipitation is therefore more specific than the conventional precipitation methods. As the remaining behind of small amounts of Ba pregnanediol glucuronide in crystalline form in the inside of the pores of the silica gel cannot be prevented, and in addition a further part simply remains adsorbed on the silica gel without other reactions and another part is contained still dissolved in the extract, a certain loss of pregnanediol glucuronide arises in the final results, which in turn is to be considered as a component of the total threshold value.

The addition of N/1000 HCl and gasoline to the extract after precipitation has taken place serves to accelerate the deposition and to avoid the adsorption of impurities on the precipitate. Owing to the extraction of the dye formed in the glucuronic acid reaction according to Tollens by means of benzene or its homologues instead of ether, the test becomes more specific. As a result of the green colouration of the benzene, weakly positive results are avoided in that any only slightly red colouration is masked by the complementary colour green. Consequently, those physiological minimum values of pregnanediol glucuronide which have their cause in the conversion of deoxycorticosterone into progesterone, in a preovulat ovarian progesterone secretion and in a possibly inadequate "normal" corpus luteum activity are not determinable. As the most important part of the total threshold value, this green colouration should make possible its exact adjustment. The same effect as due to the various individual stages of the threshold value mentioned could be achieved by reduction of the volume of the body fluid of women or urine initially used in so far as that the total amounts passing through the procedure would be subject simply to the natural sensitivity limit of the determination method. Such natural sensitivity limits, however, usually have very strong "insidious" character with a wide variation zone, so that an actual threshold value point would barely be achievable in this way.

Practical experiments using the novel procedure according to the invention have afforded a significantly higher reliability than with the best conventional contraceptive agents. It has been shown that the risk of failure of this novel type of birthcontrol is lower by eight to ten times than in the previous methods.

Patent Claims:

1. Procedure for the detection of corpus luteum metabolic products in the body fluids excreted from women with the purpose of determining the non-conceptive days of the women, characterized in that at least one corpus luteum metabolic product occurring in the body fluid is determined by reactions associated with a colour change and in that measures are taken here in order only to determine the colour change in the presence of those amounts of the metabolic product which originate from a functional corpus luteum.

2. Procedure according to Claim 1, characterized in that a corpus luteum metabolic product occurring in the body fluid of the woman is reacted with formation of a dye and the detectability of this dye is reduced so much that only those amounts of the metabolic product which originate from a functional corpus luteum are detectable.

3. Procedure according to Claim 1 or 2, characterized in that a progesterone metabolic product occurring in the urine of the woman is reacted with formation of a dye and this dye is masked by the presence of a complementary dye to the extent that only those amounts of the progesterone metabolic product which originate from a functional corpus luteum are detectable.

4. Procedure according to one of Claims 1 to 3, characterized in that pregnanediol glucuronide occurring in the urine of the woman is converted into a dye in the presence of a mixture of at least two reaction substances and this is then extracted from the reaction solution with a solvent, which solvent contains such a large amount of a complementary dye that only those amounts of pregnanediol glucuronide which originate from a functional corpus luteum are detectable.

5. Procedure according to one of Claims 1 to 4, characterized in that a glucuronic acid reaction of the

pregnanediol glucuronide occurring in the urine of the woman is carried out in the presence of hydrochloric acid and naphthoresorcinol and in that in the course of this the red-violet dye formed is extracted by means of benzene which contains such a large amount of a green complementary dye that only those amounts of pregnanediol glucuronide which originate from a functional corpus luteum are detectable.

6. Device in the form of an extraction apparatus for carrying out the procedure according to one of the above claims, characterized in that an outlet tube (**3**) which has a fine outlet opening for the body fluid to be tested is removably attached at the bottom to a container (**4**) containing the body fluid to be tested, and in that a tubular container (**2**) which contains a solvent column and is connected at its lower end to an overflow tube (**2a**) is provided perpendicularly under the outlet opening, and in that finally the free opening of the overflow (**2a**) is so far below the level of the fine outlet opening that the liquid jet emerging from the fine opening reaches the top of the solvent column in the form of a succession of liquid droplets.

7. Device according to Claim 6, characterized in that the dimensions of the fine opening of the extraction apparatus are such that it lets through a liquid jet of at most 100 cc per minute, and in that the tubular container (**2**) has a diameter of at most 20 mm.

8. Diagnostic agent for carrying out the procedure according to Claim 1, characterized in that it contains at least two substance mixtures in a total volume of at most 60 cc, of which one, in contact with at least one corpus luteum metabolic product occurring in a body fluid of the woman, leads to a colour change, while the other is suitable for inhibiting the colour change such that only those amounts of the metabolic product which originate from a functional corpus luteum are detectable.

9. Diagnostic agent according to Claim 8, characterized in that it contains at least two substance mixtures, of which one, in contact with at least one progesterone metabolic product occurring in the urine of the woman, leads to the formation of a dye, while the other has a complementary colour to this, which masks this by means of addition to the dye to such an extent that only those amounts of the metabolic product which originate from a functional corpus luteum are detectable.

Publications taken into consideration:

The American Journal of Physiology, 121, V. 1, 1938, pp. 98 to 106.

Herewith 1 sheet of drawings
